## Supporting Materials and Methods

***Frizzled Motif Associated with Bone Development* (*FRZB*) Plasmid Constructs.** IMAGE clone 360887 was purchased from Invitrogen and sequenced. Based on reference sequence GenBank NM_001463.1, there was a polymorphism at position 526 representing a C to G base change. The cDNA was subcloned into pcDNA3 (Invitrogen) by using the flanking *Eco*RI sites, and the 526 polymorphism was reverted to the reference sequence (pcFRZB) by using the QuikChange XL site-directed mutagenesis kit (Stratagene). The C to G change at exon 6 [+109] was engineered by subcloning a PCR fragment between the internal *Hin*dIII site and an *Xba*I site following the stop codon (pcFRZB-Arg324Gly). Primers 5'-GAAGCTTCGTCATCTTGGACTCAG-3' and 5'-TCTAGATTAGTTGCCTGCTTGCCG-3' were used to create this fragment. The C to T polymorphism at exon 4 [+6] was introduced into pcFRZB and pcFRZB-Arg324Gly (creating pcFRZB-Arg200Trp and pcFRZB-Arg200Trp/Arg324Gly, respectively) by using primers 5'-ACAACTATGTCATTTGGGCTAAAGTTAAAGAG-3' and 5'-CTCTTTAACTTTAGCCCAAATGACATAGTTGT-3' and with the QuikChange XL kit (Stratagene) by following the manufacturer's instructions. All plasmids were sequence verified (University of California at San Diego Cancer Center Sequence Core Facility).

**Cell Transfections.** The human embryonic kidney cell line HEK293 (American Type Culture Collection) was maintained in DMEM with high glucose supplemented with 10% FBS and 100 μg/ml penicillin and 100 μg/ml streptomycin. Before transfection, cells were grown in logarithmic phase overnight in 12-well plates. At ≈50% confluence, cells were transfected by using SuperFect (Qiagen, Valencia, CA) according to the manufacturer's instructions. For luciferase assays, typically cocktails of 0.25 μg of pTOPFLASH-Luc reporter gene vector, 0.025 μg of β-galactosidase expressing plasmid pACB-Z, 0.25 μg of β-catenin expressing plasmid (kindly provided by Hans Clevers, University Medical Center, Utrecht, The Netherlands) or 0.25 μg of pUSE-Wnt1 (Santa Cruz Biotechnology), and 1 μg of expression plasmid, and carrier DNA (pcDNA3, Invitrogen), for a total of 1.525 μg per well. Control cultures were transfected with pTOPFLASH-Luc and pACB-Z (a β-galactosidase expression vector) to establish the basal levels for the luciferase and β-galactosidase assays. After 2 h, the cells were given fresh medium and incubated for 24 h. Cells were harvested 24 h after transfection, disrupted in lysis buffer, and luciferase and β-galactosidase activities were determined by using the Dual Light reporter gene assay system (Applied Biosystems) and a microtiter plate luminometer (MicroBeta TriLux, Gaithersburg, MD). The luciferase values were normalized for variations in transfection efficiency by using the β-galactosidase internal control and are expressed as fold stimulation of luciferase activity compared with the designated control cultures. All of the transfection results represent means of a minimum of three independent transfections ± SEM.

**Immunoblotting.** After removing the medium and washing the cells with PBS, whole-cell lysates were prepared by disrupting cells in lysis buffer (10 mM Tris·HCl/150 mM NaCl/5 mM EDTA/1% Triton X-100/0.1% SDS) including phosphatase and protease inhibitor cocktails. Alternatively, separate cytosol and nuclear fractions were prepared by lysing cells in HEB buffer (20 mM Hepes/1 mM KCl/1 mM EGTA/1 mM EDTA/1 mM

DTT/1% Nonidet P-40/protease and phosphatase inhibitor cocktails), and the soluble phase was separated by centrifugation. The nuclei were picked out and then further incubated in 20 mM Hepes/1 mM EGTA/1 mM EDTA/400 mM NaCl and vortexed. The residual membranes were pelleted by centrifugation, and the supernatant was removed. Each lane of an SDS/PAGE gel was loaded with 30 μg of protein. After electrophoresis, the proteins were transferred to a polyvinylidene difluoride membrane, blocked with 0.2% I-block (Tropix, Bedford, MA) containing 0.05% Tween 20 in PBS and incubated with anti-sFRP3 (R & D Systems). Horseradish peroxidase-conjugated anti-IgG (Santa Cruz Biotechnology) was used as the secondary antibody. The membranes were developed with a chemiluminescence system (ECL detection reagent, Amersham Pharmacia) and scanned. The membranes were stripped with Re-Blot Western blot recycling kit (Chemicon) and reprobed with other antibodies and actin monoclonal antibody (Sigma) as a control. Prestained molecular weight markers (Invitrogen) were used as reference.